IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Kenji Fukudome and Charles T. Esmon

Serial No.: 09/378,261 Art Unit: 1647

Filed: August 20, 1999 Examiner: S. Gucker

For: CLONING AND REGULATION OF AN ENDOTHELIAL CELL PROTEIN C/ACTIVATED C PROTEIN C RECEPTOR

Assistant Commissioner for Patents
Washington, D.C. 20231

Response to Notice to Comply With Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures, Amendment and Declaration under 37 C.F.R. § 1.821 (f)

Sir:

Responsive to the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence disclosures mailed on July 17, 2001, in the above-identified application, applicants request use of the last filed computer readable form filed in application Serial No. 08/289,699 filed August 12, 1994, which issued December 9, 1997, as U.S. Patent No. 5,695,993, as the computer readable form for the instant application, in accordance with 37 CFR 1.821(e). The sequence listing for the instant application is identical to the sequence listing for application Serial No. 08/289,699, filed August 12, 1994. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application.

U.S.S.N. 09/378,261
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Applicants enclose amendments to the specification and a clean copy of specification pages 5 and 6 amended to insert sequence ID numbers. Also enclosed is copy of the Notice to Comply with Requirements for Patent Applications Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures.

Amendment

In the Specification

Please amend the specification as follows.

B3 On page 5, line 31, following "sequence" please insert --(SEQ ID NO:1)--.

On page 5, line 32, following "sequence" please insert --(SEQ ID NO:2)--.

B4 On page 6, line 4, following "sequence" please insert --(SEQ ID NO:2)--.

B5 On page 6, line 5, following "CCD41" please insert --(SEQ ID NO:3)--.

B6 On page 6, line 5, following "CD1d" please insert --(SEQ ID NO:4)--.

B7 On page 6, line 6, following "CD1.2" please insert --(SEQ ID NO:5)--.

B8 On page 6, line 13, following "EPCR" please insert --(SEQ ID NO:2)--.

B9 On page 6, line 14, following "EPCR" please insert --(SEQ ID NO:6)--.

B10 Since the specification as filed included an initial copy of the Sequence Listing, no amendment is required to direct entry of the Sequence Listing into the specification.

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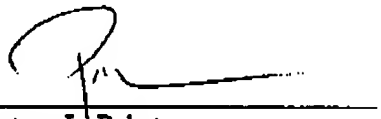
Remarks

The specification has been amended, pursuant to 37 C.F.R. § 1.821(d), to annotate the amino acid and/or nucleotide sequences disclosed in the specification.

Respectfully submitted,



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showing the time course of ^{125}I-APC binding to HUVEC. HUVEC monolayers (1.2 x 10^5 cells) were incubated at 4°C with 32 nM (filled squares) or 8 nM (open squares) ^{125}I-APC. At the indicated times, cells were washed and bound radioactivity was measured. Figure 2B is a graph of bound APC (cpm x 10^{-3}) versus unlabeled protein (nM) demonstrating the effects of unlabeled APC and rGDPC on ^{125}I-APC binding to HUVEC. HUVEC were incubated at 4°C for one hour with ^{125}I-labeled APC in the presence of the indicated concentrations (between 01 and approximately 1000 nM) of unlabeled APC (open circles) or rGDPC (closed circles). After washing, bound radioactivity was measured. Figure 2C is a graph of bound APC (fmol/well) versus free APC (nM) demonstrating the concentration dependence of ^{125}I-APC binding to HUVEC. Monolayers of HUVEC were incubated with the concentrations of ^{125}I-APC indicated as described above. Specific binding was determined as described below. Figure 2D is a Scatchard analysis of ^{125}I-APC binding to HUVEC. Each value was calculated from the data shown in Figure 2C.

Figures 3A and 3B are flow cytometric analyses of F1-APC binding to 293T cells transfected with a cDNA clone of EPCR. Cells were transfected with a clone EPCR/pEF-BOS or pEF-BOS (negative control) by the calcium/phosphate method. After 24 h, cells were harvested and F1-APC binding was performed in the absence (dotted lines) or presence of 1.3 mM CaC12 (solid lines).

Figure 4 is the predicted protein structure of EPCR based on nucleotide sequence (SEQ ID NO:1), predicted amino acid sequence (SEQ ID NO:2) and a hydropathy plot of EPCR. The signal sequence and transmembrane region are indicated with the solid bars.

Figure 5 is a comparison of the amino acid sequence of EPCR to the amino acid sequences of other members of the CD1 family and CCD41. The EPCR sequence (SEQ ID NO:2) is shown in the first line and compared to murine CCD41 (SEQ ID NO:3) (second line), human CD1d (SEQ ID NO:4) (third line) and murine CD1.2 (SEQ ID NO:5) (fourth line). Identities with EPCR are indicated by open boxes. Residues that are conserved between EPCR and all of the human CD1 family members are indicated by a double asterisk. Residues shared with one or more members of the CD1 family are indicated by a single asterisk.

Figure 6 is a comparison of the amino acid sequence of human EPCR (SEQ ID NO:2) (first line) to the amino acid sequence of murine EPCR (SEQ ID NO:6) (second line). Identities are indicated by boxes. Similarities are indicated with an asterisk.

Detailed Description of the Invention

I. Cloning and Characterization of EPCR.

Human protein C and activated protein C are shown to bind to endothelium specifically, selectively and saturably (Kd = 30 nM, 7000 sites per cell) in a Ca^{2+} dependent fashion. FL-APC binding to various human cell lines were examined, and found that the binding was HUVEC specific. A human kidney cell line transformed with SV40 large T antigen, 293T cells, expressed very few of these binding sites. A HUVEC cDNA library was constructed using the powerful mammalian expression vector, pEF-BOS (Mizushima and Nagata, (1990) Nucleic Acids Res. *18*, 5322). Plasmid DNA was prepared from subpools of independent colonies (2,500 colonies per pool), and transfected into 293T cells, using the method of Kaisho et al., (1994)

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NOTICE TO COMPLY WITH REQUIREMENTS FOR PATENT APPLICATIONS CONTAINING NUCLEOTIDE SEQUENCE AND/OR AMINO ACID SEQUENCE DISCLOSURES

The nucleotide and/or amino acid sequence disclosure contained in this application does not comply with the requirements for such a disclosure as set forth in 37 C.F.R. 1.821 - 1.825 for the following reason(s):

- [X] 1. This application clearly fails to comply with the requirements of 37 C.F.R. 1.821-1.825. Applicant's attention is directed to these regulations, published at 1114 OG 29, May 15, 1990 and at 55 FR 18230, May 1, 1990.
- [ ] 2. This application does not contain, as a separate part of the disclosure on paper copy, a "Sequence Listing" as required by 37 C.F.R. 1.821(c).
- [X] 3. A copy of the "Sequence Listing" in computer readable form has not been submitted as required by 37 C.F.R. 1.821(e).
- [ ] 4. A copy of the "Sequence Listing" in computer readable form has been submitted. However, the content of the computer readable form does not comply with the requirements of 37 C.F.R. 1.822 and/or 1.823, as indicated on the attached copy of the marked -up "Raw Sequence Listing."
- [ ] 5. The computer readable form that has been filed with this application has been found to be damaged and/or unreadable as indicated on the attached CRF Diskette Problem Report. A Substitute computer readable form must be submitted as required by 37 C.F.R. 1.825(d).
- [ ] 6. The paper copy of the "Sequence Listing" is not the same as the computer readable from of the "Sequence Listing" as required by 37 C.F.R. 1.821(e).
- [X] 7. Other: Specification needs to refer to specific SEQ ID NOs for sequences, i.e. Figures 4-6 or the brief descriptions thereof. Also, the murine sequence of Fig.6 needs its own SEQ ID NO.
- [X] An initial or substitute computer readable form (CRF) copy of the "Sequence Listing".
- [X] An initial or substitute paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification.
- [X] A statement that the content of the paper and computer readable copies are the same and, where applicable, include no new matter, as required by 37 C.F.R. 1.821(e) or 1.821(f) or 1.821(g) or 1.825(b) or 1.825(d).

For questions regarding compliance to these requirements, please contact:

For Rules Interpretation, call (703) 308-4216
For CRF Submission Help, call (703) 308-4212
For PatentIn software help, call (703) 308-6856

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